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# EVALUATION OF PATHOGENIC LEVELS OF *CYLINDROCLADIUM* AND *FUSARIUM* IN TWO STATE NURSERIES IN WISCONSIN

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## Introduction

Extensive death of white pine (*Pinus strobus* L.) in two Wisconsin state nurseries, Griffith and Wilson, prompted this evaluation. The losses were observed initially at Griffith in 2-0 beds during the 1979 growing season, and at Wilson in beds of seedlings of similar age during the 1980 season.

USDA Forest Service personnel, working cooperatively with the Wisconsin Department of Natural Resources, concluded that the mortality was caused by a root decay pathogen. Palmer and Nicholls (1980) isolated *Cylindrocladium* from the roots of diseased Griffith seedlings. Earlier, Thies (1969) documented a history of this root rot pathogen in the nursery. On the basis of this information it was decided that a quantitative survey of *Cylindrocladium* soil propagules was needed.

It appeared that the same fungus might be the cause of the decay seen at both nurseries since the development of symptoms was similar. Therefore, soils at both nurseries were screened for *Clyindrocladium* propagules. Soils at Wilson Nursery also were screened for species of *Fusarium*.

This evaluation was designed to determine if the severity of white pine root decay was correlated with populations of *Cylindrocladium* or *Fusarium* in the soil.

## Materials and Methods

Soil samples were collected in Griffith sections B-11, K-4, and A-7, and in Wilson sections B-46 and 47. Each sample was a composite of five, 6-inch cores collected with a 1-inch auger. The samples were kept cool in the field and later stored in the lab for 1 week at 5°C prior to propagule analysis.

All five sections contained or had recently contained white pine seedlings (Table 1). In 1980, approximately 50 percent of the seedlings in section B-11 were infected. Trees in section K-4 had shown damage (25 percent in 1979), but appeared to be recovering in 1980. Both of these sections had been fumigated with mylone before planting. A-7 had not been fumigated for several years and there was no root rot in white pine grown in this section and lifted in 1980. Therefore, A-7 was used to determine background levels of *Cylindrocladium* in Griffith Nursery. Before planting, Wilson Nursery sections B-46 and B-47 were fumigated with mylone (Dowfume®) and methyl bromide (MC-33®). In 1980, 2-0 white pine seedlings planted in B-46 showed damage similar to that observed at Griffith, while those in adjacent section B-47 appeared healthy.

A dilution plate technique was used to isolate Cylindrocladium from the soil. For each sample, 20 g (fresh weight) of soil was suspended in 200 ml of 0.3 percent water agar. One ml of this 1:10 dilution was plated on sucrose lima bean agar (SLBA) in triplicate (Hunter and Barnett 1978). Serial transfers were made from the 1:10 soil suspension to sterile water blanks, resulting in 1:100 and 1:1000 dilutions. One ml of each of these dilutions was plated on SLBA in triplicate. All dilution plates were incubated in the dark at 23°C for 1 week before screening for Cylindrocladium colonies. Sample colonies were transferred to pure culture for positive identification. The number of propagules/g of air-dried soil was calculated following adjustments for moisture content of the samples.

Sections B-46 and 47 soil samples also were also analyzed for the presence of Fusarium species. The methods were identical to those outlined; however, pentachloronitrobenzene (PCNB) plates (Nash and Snyder 1962) were used to determine the presence of Fusarium at 1:10, 1:500, and 1:1000 dilutions.

## Results

Cylindrocladium sp. was recovered from all of the Griffith Nursery sections examined. Cylindrocladium population levels in the recovered section, K-4, were similar to those found for the check, A-7 (Table 1). Section B-11, where symptoms were most severe, had levels much higher than the other two.

No Cylindrocladium was recovered from the initial samples at Wilson Nursery (Table 1). To confirm these findings and screen for other potential root pathogens, a second set of samples was examined. No Cylindrocladium spp. were found; however, F. solani (Mart.) App. & Wr. Snyd. & Hans. was recovered from the mylone-treated section, B-46 em. (Table 2). Although the propagule counts were relatively high, only 7 percent of the B-46 samples had colonies of this fungus.

## Discussion

The number of Cylindrocladium propagules that constitute a threshold for damage to pine seedlings is not well established. The literature suggests 8-10 propagules/g of soil (Thies 1969). However, these counts are based on only one type of propagule, the microsclerotium. Our evaluation covers all possible propagule types (conidia and hyphal fragments as well as microsclerotia). Therefore, we expect the threshold for our evaluation method to be higher than the one outlined previously.

The values of 15 and 17 propagules/g of soil for A-7 and K-4, respectively, appear to represent the background level at Griffith. Neither of these sections was severely damaged with root rot. However, the elevated population, 58 propagules/g of soil, in B-11 was associated with severe root decay.

An examination of the fumigation history of section B-11 points to a possible reason for the elevated levels of Cylindrocladium. A sorghum sudan cover crop was grown in B-11 before fumigation. Due to a labor dispute, the cover crop was allowed to attain a height of approximately 5 feet. This large size made it difficult to turn the soil and resulted in large clumps remaining in the soil. Fumigants cannot penetrate large clumps, which leads to incomplete fumigation. Cylindrocladium species are known to be poor competitors and tend to do well only when other soil microorganisms are limited. Incomplete fumigation would present an ideal environment for the expansion of populations of Cylindrocladium species that remain.

The results of the screening for Fusarium did not identify the cause of white pine root decay at Wilson Nursery. Although, F. solani was recovered from the beds at high rates, there are no confirmed reports of this fungus as a pathogen of conifers. F. solani has been found in association with decaying root tissue (Pawuk 1978; Pawuk and Barnett 1974; Vaartaja and Bumbieris 1967), but it remains unclear whether the fungus acts as a primary or secondary pathogen or as a saprophyte.

## Conclusions

1. The results of this evaluation, when considered with the findings of Palmer and Nicholls (1980), suggest that a Cylindrocladium species is the cause of the white pine root rot at Griffith Nursery.

2. High populations of Cylindrocladium probably developed as a result of incomplete soil fumigation.

3. Although Fusarium solani was recovered, the cause of the white pine root decay at Wilson State Nursery has not been determined.

## Recommendations

When soil fumigants are applied, care should be taken to follow label recommendations for prefumigation soil preparation. Application rates should be followed strictly to ensure consistent results.

## Pesticide Precautionary Statement

The pesticides reported on and recommended here were registered for the use described at the time this manuscript was prepared. Since the registration of pesticides is under constant review by State and Federal authorities, a responsible State agency should be consulted as to the current status of this pesticide.

Pesticides can be injurious to humans, domestic animals, desirable plants, and fish or other wildlife--if they are not handled or applied properly. Use all pesticides selectively and carefully. Follow recommended practices for the disposal of surplus pesticides and pesticide containers.

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Table 1.-- Results of soil dilution analysis to determine presence or absence of *Cylindrocladium* spp. at Wilson and Griffith State Nurseries.

| Nursery | Plot location | Fumigant | Symptom expression | No. samples examined | Percent samples with colonies | No. Progagules/g of soil |
|---|---|---|---|---|---|---|
| Griffith | B-11 | Mylone | Present | 27 | 48 | 58 |
| Griffith | K-4 | Mylone | Recovered | 24 | 29 | 17 |
| Griffith | A-7 | None | Absent | 4 | 25 | 15 |
| Wilson | B-46 | Mylone | Present | 15 | 0 | 0 |
| Wilson | B-47 | MC-33 | Absent | 15 | 0 | 0 |

Table 2.-- Results of soil dilution analysis to determine presence or absence of *Cylindrocladium* spp. and *Fusarium* spp. at Wilson State Nursery.

| Fungus | Plot location | Fumigant | Symptom expression | No. samples examined | Percent samples with colonies | No. propagules/g of soil |
|---|---|---|---|---|---|---|
| *Cylindrocladium* spp. | B-46 | Mylone | Present | 20 | 0 | 0 |
| *Cylindrocladium* spp. | B-47 | MC-33 | Absent | 10 | 0 | 0 |
| *Fusarium solani* | B-46 | Mylone | Present | 20 | 7 | 5020 |
| *F. solani* | B-47 | MC-33 | Absent | 10 | 0 | 0 |